

WHAT IS CLAIMED IS:

1. A method for the identification of interacting protein, said method comprising:
 - a) subjecting an extract to protein-affinity chromatography on multiple columns, said columns having a protein ligand coupled to the column matrix in varying concentrations, and eluting bound components of said extract from said columns;
 - b) separating said components to isolate an interacting protein;
 - c) analyzing the interacting protein by mass spectrometry to identify the interacting protein.
- 10 2. The method of claim 1, wherein said columns are micro-columns.
3. The method of claim 1, wherein said separation is a gel-separation.
- 15 4. The method of claim 3, wherein said gel-separation is a polyacrylamide gel electrophoresis.
5. The method of claim 4, wherein said polyacrylamide gel does not contain SDS.
- 20 6. The method of claim 1, wherein said protein ligand is covalently bound to the matrix.
7. The method of claim 1, wherein said mass spectrometry is MALDI-TOF mass spectrometry.
- 25 8. The method of claim 1, wherein the bound components of the extract are eluted with a protein denaturant
9. A method for the identification of an interacting protein, said method comprising:
 - a) subjecting a cellular extract or extracellular fluid to protein-affinity chromatography on multiple columns, said columns having a protein ligand coupled to the column matrix in varying concentrations, and eluting bound components of said extract from said columns;

- 10 b) gel-separating said components to isolate an interacting protein; wherein the
interacting protein is observed to vary in amount in direct relation to the
concentration of coupled protein ligand;
5 c) digestion of said interacting protein to give corresponding peptides
d) analyzing said peptides by MALDI-TOF mass spectrometry or post source decay
to determine the peptide masses, and
e) correlative database searching with said peptide or peptide fragment masses,
whereby the interacting protein is identified

- 10 10. The method of claim 9, wherein said columns are micro-columns.
11. The method of claim 9, wherein said gel-separation is a polyacrylamide gel
electrophoresis.
- 15 12. The method of claim 11, wherein said polyacrylamide gel does not contain SDS.
13. The method of claim 9, wherein said protein ligand is covalently bound to the matrix.
- 20 14. The method of claim 9, wherein the identities of the interacting protein partners are
entered into a relational database.
- 25 15. The method of claim 9, wherein the bound components of the extract are eluted with a
protein denaturant.

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